Flavin adenine dinucleotide (FAD) hydrolysis in cow and human milk

Time in h	Amount of FAD (μg)		Amount of riboflavin and riboflavin phosphate produced and calculated as riboflavin (μg)		Percentage of FAD destruction according to riboflavin estimation	
	Cow	Human	Cow	Human	Cow	Human
0	10.0	10.0	13.08	10.25	–	–
1			18.91	13.91	76.4	53.0
2			19.00	14.82	78.0	62.0
3			19.00	14.82	78.0	62.0

concentrated material, Butanol/acetic acid/H_2O system of CRAMMER[4] was used. FAD was enzymatically estimated in the D-amino acid oxidase system as described by MANSON and MODI[5]. Under these conditions, 0.5 μg FAD produced an oxygen uptake of 75 μl; on examining 2 ml of whole milk for the presence of free FAD no detectable amount was found to be present. On the other hand, when human milk was boiled for 3 min, the supernatant free from precipitated proteins contained FAD. In analyzing a number of such samples, it was observed that the FAD content varied between 0.15 and 0.4 μg/ml. On paper chromatographic examination, one fluorescent spot corresponding to FAD was seen. From the above observation, it seems that FAD of human milk is bound to one or more of the milk proteins which on heating become denatured and liberate free FAD.

The complete absence of free FAD cannot, however, be assumed, because the method, due to the limitations of the assay system, could be expected to detect only amounts greater than 0.02 μg/ml. In order to explore the possibility of the occurence of free FAD in human milk, the fate of FAD added to raw milk was studied. MANSON and MODI[5] had observed 100% degradation of FAD in cow milk within 1 h. 2 ml samples of cow and human milk were incubated at 37°C with 20 μg FAD for 3 h. The amount of FAD present in trichloroacetic extracts was estimated by the method of BURCH, BESSEY and LOWRY[6]. From the figures listed in the Table, it can be seen that, though less than in cow milk, there was considerable destruction of FAD in human milk. Paper chromatography indicated that FAD was hydrolysed to free riboflavin. This was further confirmed by incubating known amounts of FAD with human milk proteins obtained by ammonium sulphate fractionation. The results indicate that, if 0.2 μg of FAD was incubated with milk protein and the specific protein of D-amino acid oxidase, wide variations in oxygen uptake were obtained depending upon the order in which the reactions were added to the incubation vessel. When FAD and enzyme solution were mixed before the addition of milk fraction, concordant values for the rate of O_2 consumption were always obtained. In this experiment it was observed that milk proteins hydrolyzed the added FAD, thus suppressing the reaction of D-amino acid oxidase.

Preliminary studies have been made to characterize the combined FAD form. Cow milk is a rich source of xanthine oxidase, of which FAD is the prosthetic group. Unlike cow milk, human milk does not contain xanthine oxidase[1], or, if it does contain it, is a very poor source[7]. Therefore, a preliminary study about the occurrence of milk flavoproteins was made. Using the procedure of BAILLE and MORTON[8], reduced diphosphopyridine nucleotide-diaphorase and reduced diphosphopyridine nucleotide cytochrome C reductase in human milk were detected in significant amounts, whereas glycine oxidase and succinic dehydrogenase were not detectable, using the method of RATNER[9] and BONNER[10] respectively.

Zusammenfassung. Riboflavin ist in der Muttermilch in Form von Flavin-adenin-dinucleotid anwesend, was für eine Komplexform spricht. Ferner wurden in der Milch zwei Flavoproteine, die DPN-Cytochrom C-Reduktase und DPN-Diaphorase reduzierten, gefunden.

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Oxygen Isotope Paleotemperature Measurements on Lower Jurassic Belemnoidea from Bamberg (Bavaria, Germany)

Specimens of Belemnoidea were collected from the Trimeusel locality on the Main river near Bamberg (Bavaria)[1]. This is an outcrop of the upper part of Lias epsilon (Toarcian). The oxygen isotope analyses were carried out on CO_2 samples prepared according to the normal techniques[2] and using an Atlas-Werke M86 mass-spectrometer. The standard was CO_2 made from Carrara marble, but the results reported are corrected to relate to

[1] The specimens were kindly donated by Dr. D. H. WELTE of the Geologisch-Paläontologisches Institut der Universität Würzburg.
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PDB-1 Chicago. Three specimens were unaltered and gave reproducible results. The data are as follows:

1. Most negative specimen: Sample 1: $\delta = -2.59^0/_{00}$
Sample 2: $\delta = -2.54^0/_{00}$
Sample 3: $\delta = -2.52^0/_{00}$
Average: $\delta = -2.55^0/_{00}$

2. Sample 1: $\delta = -2.30^0/_{00}$
Sample 2: $\delta = -2.30^0/_{00}$
Sample 3: $\delta = -2.19^0/_{00}$
Average: $\delta = -2.26^0/_{00}$

3. Sample 1: $\delta = -1.75^0/_{00}$
Sample 2: $\delta = -1.70^0/_{00}$
Sample 3: $\delta = -1.65^0/_{00}$
Average: $\delta = -1.70^0/_{00}$

Assuming the mean ocean δ to have been zero (as at present), the paleotemperatures corresponding to the average values listed are as follows:

Specimen 1: 28.4°C
Specimen 2: 27.0°C
Specimen 3: 24.2°C

The significance of these results is apparent when they are compared with others obtained by the author elsewhere in Europe[3]. From the Toarcian (upper Lias) of Yorkshire and Northampton temperatures of 31.7°C and 29.6°C, 25.7°C, 23.8°C respectively were obtained. A specimen of the same age from Thouars in France gave a temperature of 24.9°C, while two other specimens from the Pliensbachian of St. Vincent Herlanges gave temperatures of 24.6°C and 24.3°C respectively. Measurements were made on three Toarcian Belemnoidea from Switzerland. One, from Sulz (Fricktal, Kanton Aargau), gave a temperature of 27.3°C. A second, from Les Pueys (Canton de Fribourg), gave 23.5°C. The third, from Le Tabousset (Canton de Vaud), gave 23.4°C. The data show a considerable uniformity of temperature to have existed throughout Western Europe in Liassic times. They also show that this was a high temperature and this can be demonstrated too by comparing them with temperatures recorded from, e.g., the Middle Jurassic of France (range: 19.5°C to 21.8°C) and the Upper Jurassic of Germany (range: 20.5°C to 21.8°C)[3]. The Toarcian and Pliensbachian emerge as very warm, equable time intervals. The scarcity of limestones and coral reefs at this time, however, has led some workers[4] to infer cooler conditions than in the later Jurassic. That this conclusion is erroneous is demonstrated empirically by the oxygen isotype analyses, but it can be shown to be mistaken on other grounds. The formation of limestone depends on temperature and also on the amount of inorganic clastics being introduced into the area of potential limestone formation. Thus limestone, even under optimum temperature conditions, may not form because of excessive dilution by influx of non-calcareous sediment[5]. An interesting point in this connection is that at least in one case, a Caribbean core, carbonate content and temperature are unrelated[6]. In addition, it is worth mentioning the rich flora of temperate facies found in the early Lias of East Greenland[7]. The universal distribution of Ammonoid faunas in the Lower Jurassic leads to the inference that the high temperatures found so uniformly in the upper Lias probably existed through the rest of the Lias as well. Further analyses are necessary to demonstrate this physico-chemically.

Zusammenfassung. Belemniten aus dem Lias epsilon von Trimeusel bei Bamberg (Bayern) wurden mit Hilfe von Sauerstoffisotopen analysiert und die Palaeotemperatur bestimmt. Vergleiche mit Belemniten aus dem unteren Lias diverser europäischer Gebiete ergaben den Nachweis einer Periode mit warmen Meerwasser. Dies steht im Gegensatz zu Angaben anderer Autoren, die auf dem Fehlen von Kalkablagerungen und Korallenriffen basierten.

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Structure of the Nephridia of the Indian Cattle Leech *Hirudinaria granulosa* (Savigny) with Remarks on their Nephridial Microflora

BHATIA[1] confused the bacteria present in the nephridial bladders of *Hirudinaria granulosa* with cilia and described their size, distribution, and vibratility. In fact the fluid, filling the nephridial bladders, was turbid in appearance due to excessive bacterial growth in it. The bacilli were mostly non-motile (vibratility being due to the Brownian movement), 2.8 to 7 μ (average 4 to 5 μ) in length, Gram-negative, non-acid fast and formed macroscopic clumps. The vesicles were internally lined with a whitish layer of the same type of bacilli, which could be removed by means of a brush. The layer was thickest in the upper part of the vesicle and its thickness diminished towards the nepridiopore. On the postero-dorsal side of each vesicle was present a whitish patch, formed of a comparatively better growth of bacteria, coinciding with the opening of the vesicle-duct into the nephridial bladder. Sections of the vesicles, stained with Gram's technique[2,3] using Hucker-Conn crystal violet, Weigert's iodine, and Saffranin, showed an uneven internal layer of Gram-negative bacilli.

The bacteria were counted by using the haemocytometer. The fluid was diluted so as to contain about 10^7 to 10^8 cells per ml for accurate counting, thoroughly churned in order to break the bacterial clumps, filled in the counting chamber and counting done under dark-ground illumination. The fluid contained 6.5 to 10.6 million bacterial cells per cm^3 (average 8.1 million).

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